

U.S. Patent Application Serial No. 10/822,860
Amendment filed March 9, 2007
Reply to OA dated December 14, 2006

AMENDMENTS TO THE CLAIMS:

Please amend claims 1, 2, 4 and 5, and add new claims 13 and 14, as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (Currently Amended): ~~Polyclonal~~ An affinity-purified polyclonal antibody specific for a phosphorylated linker region in Smad2 and/or Smad3.

Claim 2 (Currently Amended): ~~The~~ A polyclonal antibody ~~according to claim 1~~ specific for a phosphorylated linker region in Smad2 and/or Smad3, obtained from antiserum raised by immunizing a mammal with a phosphorylated product of a peptide including an amino acid sequence in the linker region of Smad2 or Smad3.

Claim 3 (Original): The polyclonal antibody according to claim 2, wherein the phosphorylated product of a peptide including the amino acid sequence in the linker region of Smad2 for the immunization is:

Pro Ala Glu Leu p-Ser Pro Thr Thr Leu p-Ser Pro Val Asn His Ser

(SEQ ID NO: 1)

wherein p-Ser represents phosphorylated serine

and

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the phosphorylated product of a peptide including the amino acid sequence of the linker region of Smad3 for the immunization is:

Ala Gly Ser Pro Asn Leu p-Ser Pro Asn Pro Met p-Ser Pro Ala

(SEQ ID NO 2)

wherein p-Ser represents phosphorylated serine.

Claim 4 (Currently Amended): The polyclonal antibody according to ~~any one of claims 1 to 3~~ claim 2, wherein the mammal is a rabbit.

Claim 5 (Currently Amended): The polyclonal antibody according to ~~any one of claims 1 to 3~~ claim 2, wherein the raised antiserum is affinity purified with a phosphorylated peptide(s).

Claim 6 (Withdrawn): Use of polyclonal antibody according to any one of claims 1 to 3 in screening of drugs that inhibit phosphorylation of the linker region in Smad2 or Smad3.

Claim 7 (Withdrawn): A method of screening drugs that inhibit phosphorylation of the linker region in Smad2 or Smad3, including the steps of:

- (i) bringing mammalian cells, in which TGF-b receptor is intrinsically expressed or overexpressed, into contact with a candidate drug before treating the cells with TGF-b;
- (ii) incubating the cells together with TGF-b;

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(iii) recovering and homogenizing the cells after the incubation to obtain a homogenate;
(iv) incubating the obtained homogenate together with an antibody(ies) specific for a Smad protein(s) to form an immunoprecipitate; and
(v) detecting the presence or absence of a phosphorylated Smad protein(s) by reacting the immunoprecipitate with the polyclonal antibody according to any one of claims 1 to 6 to infer the inhibition of phosphorylation.

Claim 8 (Withdrawn): The method according to claim 7, wherein the drug is an anti-fibrosis drug.

Claim 9 (Withdrawn): A method of screening drugs that inhibit phosphorylation of a Smad protein(s), including the steps of:

(i) bringing a Smad protein(s), as a substrate(s), into contact with a candidate drug;
(ii) reacting said Smad protein(s) with active p38 in the presence of ATP; and
(iii) detecting phosphorylated Smad protein(s) in the reacted Smad protein(s) to evaluate the inhibition of phosphorylation.

Claim 10 (Withdrawn): A method of screening drugs that inhibit phosphorylation of a Smad protein(s), including the steps of:

(i) stimulating arbitrary cells with TGF- β and recovering the cells after a predetermined time;

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(ii) immunoprecipitating a homogenate of the recovered cells with an antibody(ies) specific for a kinase;

(iii) incubating the immunoprecipitated samples, a candidate drug(s) recombinant Smad2 and recombinant Smad3 and phosphorylating Smad2 and Smad3 in vitro; and

(iv) detecting a phosphorylated Smad protein(s) in the reacted Smad proteins by immunoblotting technique using an antibody(ies) against phosphorylation in the linker region to evaluate the inhibition of phosphorylation.

Claim 11 (Withdrawn): A method for assessing the activity of fibrosis stimulating signal in hepatic fibrosis and the efficacy of the molecular targeting therapy for hepatic-fibrosis, in which the antibody according to any one of claims 1 to 3 is incubated with a sample of object tissue, comprising the steps of:

(i) collecting a tissue of affected regions of a patient with hepatic fibrosis and of a drug-treated patient with hepatic fibrosis and preparing a tissue specimen slice from the collected tissue pieces;

(ii) fixing the prepared tissue specimen slice on a plate for exclusive use and reacting it with a blocking solution for blocking non-specific reactions of the antibodies;

(iii) incubating the tissue specimen slice having been reacted with the blocking solution with the antibody according to any one of claims 1 to 5 which is specific for the phosphorylation in the linker region;

(iv) washing the tissue specimens having been reacted with the antibody and further incubating the tissue specimen slice with a secondary antibody labeled with an enzyme; and

(v) detecting the signal with a detecting reagent and assessing the activity of the fibrosis stimulating single and the efficacy of the drug used in molecular targeting therapy based on the intensity of the signal corresponding to a phosphorylated Smad(s).

Claim 12 (Withdrawn): A method for assessing the activity of oncogenesis stimulating signal in human colon cancer and the efficacy of the molecular targeting therapy for human colon cancer, in which the antibody according to any one of claims 1 to 3 is incubated with a sample of object tissue, comprising the steps of:

(i) collecting a tissue of affected regions of a patient with colon cancer and of a drug-treated patient with colon cancer and preparing a tissue specimen slice from the collected tissue pieces;

(ii) fixing the prepared tissue specimen slice on a plate for exclusive use and reacting it with a blocking solution for blocking non-specific reactions of the antibody;

(iii) incubating the tissue specimen slice having been reacted with the blocking solution with the antibody according to any one of claims 1 to 5 which is specific for the phosphorylation in the linker region;

(iv) washing the tissue specimen slice having been reacted with the antibody and further incubating the tissue specimen slice with a secondary antibody labeled with an enzyme; and

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(v) detecting the signal with a detecting reagent and assessing the activities of the oncogenesis stimulating signal and the efficacy of the drug used in molecular targeting therapy based on the intensity of the signal corresponding to a phosphorylated Smad(s).

Claim 13 (New): The polyclonal antibody according to claim 3, wherein the mammal is a rabbit.

Claim 14 (New): The polyclonal antibody according to claim 3, wherein the raised antiserum is affinity purified with a phosphorylated peptide(s).